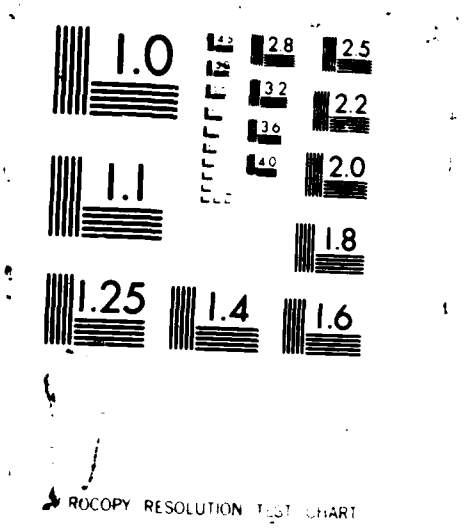
AD-A185 969 IMMUNOLOGY OF CHROMOGRANIN CONTAINING SPLENOCYTES(U) 1/1
PENNSYLVANIA UNIV PHILADELPHIA W F HICKEY 15 SEP 87
N00014-86-K-0457

UNCLASSIFIED F/G 6/5 NL


1.0
1.1
1.25
1.4
1.6
1.8
2.0
2.2
2.5
2.8
3.2
3.6
4.0
ROCOPY RESOLUTION TEST CHART

(U)

(2)

AD-A185 969 DTIC

REPORT DOCUMENTATION PAGE

DTIC ELECTE OCT 16 1987 S D

DTIC FILE COPY

1a. REPORT SECURITY CLASSIFICATION: (U)	1b. RESTRICTIVE MARKINGS: (NA)
2a. SECURITY CLASSIFICATION AUTHORITY: (NA)	3. DISTRIBUTION / AVAILABILITY OF REPORT: unlimited
2b. DECLASSIFICATION / DOWNGRADING SCHEDULE: (NA)	
4. PERFORMING ORGANIZATION REPORT NUMBER(S): University of Pennsylvania	5. MONITORING ORGANIZATION REPORT NUMBER(S): (NA)
6a. NAME OF PERFORMING ORGANIZATION: University of Pennsylvania; 6b. OFFICE SYMBOL (If applicable): (NA)	7a. NAME OF MONITORING ORGANIZATION: Office of Naval Research
6c. ADDRESS (City, State, and ZIP Code): Univ. of Pennsylvania, Neuropathology - 454 Johnson Pavilion, Philadelphia, PA 19104-6179	7b. ADDRESS (City, State, and ZIP Code): 800 N. Quincy Avenue, Arlington, VA 22217-5000
8a. NAME OF FUNDING / SPONSORING ORGANIZATION: Office of Naval Research; 8b. OFFICE SYMBOL (If applicable): ONR	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER: N00014-86-K-0457
8c. ADDRESS (City, State, and ZIP Code): 800 N. Quincy Avenue, Arlington, VA 22217-5000	10. SOURCE OF FUNDING NUMBERS: PROGRAM ELEMENT NO. 61153N; PROJECT NO. RR04108; TASK NO. -; WORK UNIT ACCESSION NO -;

11. TITLE (Include Security Classification)

Immunology of Chromogranin Containing Splenocytes

12. PERSONAL AUTHOR(S)

William F. Hickey, MD

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
FINAL	FROM 7/86 TO 9/87	87-Sept-15	4

16. SUPPLEMENTARY NOTATION

(NA)

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	Chromogranin, leukocyte, neuroendocrine

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

During the funding period this laboratory has continued studies aimed at defining a role for a novel splenocyte. These investigations have involved extensive phenotypic analyses and in vitro functional assays in well defined test systems. To date our results have been consistent with the hypothesis that the chromogranin containing splenocyte population represents a heretofore undefined leukocyte. However, functional studies have not yet revealed a system in which this cell exerts a detectable effect. Attempts to carry this cell type long term in vitro have been unsuccessful.

20. DISTRIBUTION / AVAILABILITY OF ABSTRACT: ☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT. ☐ DTIC USERS	21. ABSTRACT SECURITY CLASSIFICATION: (U)
22a. NAME OF RESPONSIBLE INDIVIDUAL: Dr. J. A. Madje	22b. TELEPHONE (include Area Code): 202-696-4055; 22c. OFFICE SYMBOL: ONR

DD FORM 1473, 84 MAR 83 APR edition may be used until exhausted. All other editions are obsolete.

SECURITY CLASSIFICATION OF THIS PAGE

FINAL REPORT*

IMMUNOLOGICAL EFFECTS OF CHROMOGRANIN CONTAINING SPLENOCYTES
1-JULY-1986 TO 30-SEPT-1987
ONR Contract N00014-86-K-0457

Introduction:

The purpose of this grant was to characterize, isolate and detect a function for an apparently new type of cell in the immune system. In this endeavor multiple phenotypic studies and known immunological assays for cell function have been employed attempting to define the uniqueness of this chromogranin containing cell. Since chromogranin is a marker protein for members of the diffuse neuroendocrine system, the members of which exert marked effects on homeostatic mechanisms of an animal, it is hoped that an immunoregulatory role of some type will be identified for this cell. The data accumulated to date support its unique nature in that it is phenotypically and morphologically dissimilar to any known member of the hematopoetic/reticulo-endothelial/immunological systems. However, there has been no detection of activity in any of the assays employed.

Phenotyping:

Cells have been isolated from neonatal rat spleen employing a fluorescence-activated cell sorter. The results of phenotypic studies employing well defined antibodies against cell surface antigens are shown in Table I. These findings require some elaboration to establish the chromogranin containing cell as a novel cell type. Cells of the spleen are of many diverse types, and it is best to enumerate and eliminate them one by one. T-lymphocytes of the rat are positive for the antibodies W3/13, OX-19 and OX-7; the cell we have identified has only the first of these surface antigens, W3/13. This antigen is also present on the surface of granulocytes and in brain tissue. The absence of the latter two markers is strong evidence it is not a typical member of the T-lymphocyte family. In addition, it is negative for OX-22, W3/25 and OX-8 which identify T-cell subsets including cells of the rat NK variety. These cells are negative for ED-1 and ED-2 which detect monocyte/macrophage/dendritic types of cells involved in antigen processing and presentation, thus removing them from that family of cells. They are mildly positive for the OX-42 marker however, which detects tissue macrophages, monocytes and some granulocytes. This finding is of interest in that is suggests that they may have some similarities to antigen presenting cells, albeit remote, or they may be a granulocytic cell type. They are negative for cell surface Ia molecules, a necessity for class II restricted antigen presenting cells, but they are moderately positive for the MHC class I molecule which can be used as a restriction element in some forms of antigen presentation. Finally, they are negative for cell surface immunoglobulin light chains and the OX-33 marker, thus

* This contract has been electively terminated after 15 months due to the relocation of the investigator.

ruling out their identity with B-cells or plasma cells. That they are leukocytes and members of the immune system's repertoire of cells is nonetheless supported by their OX-1 and asialo-GM1 positivity.

Are these cells some type of granulocyte? Some of the antibodies employed cross react with granulocytes (W3/13, OX-42, OX-1) and the chromogranin containing cells are positive to variable degrees with these probes. Morphologic studies of the purified chromograinin positive splenocytes permit us to rule out the possibility that these cells are any of the three typical granulocytic forms - neutrophil, eosinophilic, or basophil/mast cell. They contain scattered, simple membrane bound granules not typical in number or morphology for any of those cell types. It is however a possibility that they represent an immature or developmental form of one of these lines. Their morphology is indeed similar to the promyelocyte stage of granulocytic development, and to the immature form of basophils.

To eliminate this first possibility bone marrow cells were enriched for promyelocytic cell forms by 48 hour culture with colony stimulating factor which promotes the proliferation and differentiation of such cells. Immunoblot analysis of the resulting cells rich in promyelocytes demonstrated that they were negative for chromogranin which our cells contain. In addition, promyelocytes have been reported to be Thy-1 positive on their surface, these cells are not. Finally, we examined promyelocytes for a number of patients with promyelocytic leukemia - a human disease in which large numbers of promyelocytes are present in the circulation. Once again, promyelocytes were negative for chromogranin. Thus we feel confident that this cell is not merely a promyelocyte.

It is more difficult to exclude the possibility that this cell may be an immature mast cell. Currently we are examining these chromogranin positive splenocytes for the presence of high affinity IgE cell surface receptors; immature mast cells do possess these. Should the cells we have purified be negative for this molecule, we will feel confident that this cell is indeed a unique type of leukocyte.

The justification for such an extensive phenotypic characterization rests on the fact that cells of the immune system and the spleen have been extensively studied over many years. In order to claim novelty without an identified unique function requires that we prove dissimilarity between this splenocyte and all other defined cell types.

All of the data given in outline form above is in manuscript form awaiting only the information on cell surface IgE receptors. If these cells prove negative for them, then the manuscript will be submitted for publication as reporting the identification of a novel cell type.

Functional Assays:

In an attempt to define a function or response of these cells, we have employed a number of standard assays, unfortunately with only negative results. Semipurified chromogranin containing spleen cells were stimulated by the lectins concanavalin-A and phytohemagglutinin as well as with bacterial lipopolysaccharide. Their proliferative response was measured by using a standard ^{3}H-thymidine incorporation assay. The cells did not show enhanced thymidine incorporation or proliferation in response to any of these.

A-1

The chromogranin containing cells were also examined for any effect they might exert on an MHC class II dependent antigen proliferation response. In this study rat T-helper cells specific for myelin basic protein were pulsed with varying concentrations of antigen in the presence of Ia positive accessory cells. The antigen dependent proliferative response was measured by ^{3}H-thymidine incorporation. This study was performed in duplicate with the chromograninin cells in the test wells of half the assay. No effect attributable to these cells was detected.

Finally, these cells were analyzed for any effect they might exert on the Interleukin-2 dependent expansion of stimulated T-lymphocytes. Again, by incorporation of radioactive thymidine no difference was found between pools of T-lymphocytes with chromogranin cells being present during IL-2 driven expansion and those without these cells.

As is evident from the above information, to date no immunoregulatory role for the chromogranin containing splenocytes has yet been identified. I have concentrated on T-lymphocyte systems because they are routinely used in this laboratory. In the future it will be necessary to examine the effect (if any) on B-cell stimulation, antibody production, and Killer cell systems.

Scheduled Studies:

A six month hiatus is necessary in the research proposed in the initial grant application because of this investigator's relocation to Washington University in Saint Louis. Upon resumption of laboratory activities animal studies will begin in which rats are given opiate, parasympathomimetic or sympathomimetic agents for five days. After these periods of treatment the spleen will be analyzed by immunohistochemestry and compared to controls to identify any quantitative increase or decrease per unit area of chromogranin positive cells. Since the diffuse neuroendocrine system and the immune system both respond to various neural stimuli and neurological states mimiced by these drugs, it is hoped that an effect on the cells can be identified. The details of this study were elaborated in the initial grant application.

New Directions:

Since no function for the chromogranin containing cells of the spleen has yet been identified, one logical step is to attempt to identify the substances which their granules contain. In my new location at Washington University I will be working in association with Dr. Kevin Roth who has specialized in the study of endogenous opiods and other neurotransmitter like peptides known to be active on cells of the immune system. He has agreed to assist me in the examination of the Chromogranin containing splenocytes for a number of these substances. This will be an important addition to the approaches already taken since it may permit the identification of an immunological system in which the cells may be active based upon the type of substances the cell's granules contain.

TABLE I

PHENOTYPE OF CHROMOGRANIN CONTAINING SPLENOCYTES

Antibody	Specificity	Results (%)*
OX-1	Leukocyte common antigen	++++ (96)
OX-6	rat MHC Class II (Ia)	- (3)
OX-7	Thy 1.1 molecule - most thymic T-cells and circulating T-lymphocytes, also CNS tissue.	- (4)
W3/13	Peripheral T-cells, granulocytes and CNS tissue	++++ (96)
W3/25	T-lymphocytes of the helper phenotype CD-4 molecule, also activated macrophages	- (2)
OX-8	T-lymphocytes with suppressor/cytotoxic function, also rat NK cells	- (2)
OX-18	Rat class I MHC antigen	++ (40)@
OX-19	Rat pan-T lymphocyte marker	- (2)
OX-22	T-lymphocyte antigen found on a subset of of helper and suppressor cells	- (3)
OX-42	Monocytes/macrophages, granulocytes and microglia	++ (35)@
antiserum	Asialo-GM_1	++++ (99)
MAR 18.5	Rat immunoglobulin light chain	-#
OX-33	Rat B-lymphocytes	-#
ED-1	cytoplasmic and surface antigen of macrophages and monocytes	-#
ED-2	Cell membrane antigen of subsets of tissue macrophages and monocytes	-#

*The percentages given represent the portion of the cell population falling beyond the negative control profile. In all the studies the CRG positive cells distributed as a single peak. @ An intermediate level of positivity (++) probably represents a spectrum of positivity of all cells in the peak although some are dim and overlap with the negative. # These profiles were run on a different FACS machine for qualitative not quantitative results - all were negative.

INDEX:

Papers on Chromogranin during this period:

Quan J, Hickey WF, Hogue-Angeletti R: Neuroendocrine cells of the lamina propria; J. Neuroimmunol. (in press)

Hickey WF, Prystowski MB, Hogue-Angeletti R: Isolation of a novel splenocyte containing chromogranin; (manuscript in preparation)

Hogue-Angeletti R, Hickey WF: Neuroendocrine cells within immune tissues; Annals N.Y. Academy of Sci.; 496:78, 1987.

END

DATE

FILMED

DEC.

1987